

[NAME OF DOCUMENT] CLAIMS

[Claim 1] A method for preparing a cell concentrate, which comprises: introducing a cell-containing solution that contains nucleated cells and unnecessary cells into a filter device comprising a filter material for substantially capturing the nucleated cells and for substantially giving passage to the unnecessary cells, so as to capture the nucleated cells by the above-described filter material and to discharge the unnecessary cells from the above device; and introducing a recovery solution into the above-described filter device, so as to recover the nucleated cells captured by the above-described filter material,

wherein the above-described method is characterized in that the cell-containing solution that contains nucleated cells and unnecessary cells are separated into a layer that is rich in nucleated cells and a layer that is rich in unnecessary cells, the layer rich in unnecessary cells is first introduced into the above-described filter device, and the layer rich in nucleated cells is then introduced therein, so as to discharge the unnecessary cells remaining in the above-described filter device while capturing the nucleated cells by the above-described filter material, and a recovery solution is then introduced into the above-described filter device, so as to recover the nucleated cells captured by the above-described filter material.

[Claim 2] The method for preparing a cell concentrate according to claim 1, wherein unnecessary cells are precipitated by gravity or centrifugal force, so as to separate a cell-containing solution that contains at least nucleated cells and unnecessary cells into a layer that is rich in nucleated cells and a layer that is rich in unnecessary cells.

[Claim 3] The method for preparing a cell concentrate according to claim 1, wherein unnecessary cells are agglutinated and are then precipitated by gravity or centrifugal force, so as to separate a cell-containing solution that contains at least nucleated cells and unnecessary cells into a layer that is rich in nucleated cells and a layer that is rich in unnecessary cells.

[Claim 4] The method for preparing a cell concentrate according to any one of claims 1 to 3, wherein the above-described unnecessary cells are erythrocytes.

[Claim 5] The method for preparing a cell concentrate according to any one of claims 1 to 4, wherein the above-described nucleated cells are hematopoietic stem cells.

[Claim 6] The method for preparing a cell concentrate according to claim 3, wherein unnecessary cells are agglutinated by adding hydroxyethyl starch to a cell-containing solution that contains nucleated cells and unnecessary cells.

[Claim 7] The method for preparing a cell concentrate according to any one of claims 1 to 6, wherein the above-described layer that is rich in nucleated cells consists

of a nucleated cell-concentrated layer and a nucleated cell-diluted layer, and wherein the nucleated cell-diluted layer and the nucleated cell-concentrated layer are introduced into the filter device in this order.

[Claim 8] The method for preparing a cell concentrate according to any one of claims 1 to 5, wherein the above-described layer that is rich in nucleated cells consists of a nucleated cell-concentrated layer and a nucleated cell-diluted layer, and wherein the nucleated cell-concentrated layer and the nucleated cell-diluted layer are introduced into the filter device in this order.

[Claim 9] The method for preparing a cell concentrate according to claim 8, wherein a part of or all of the above-described nucleated cell-diluted layer is used as at least a portion of a recovery solution.

[Claim 10] The method for preparing a cell concentrate according to any one of claims 1 to 9, wherein a recovery solution is introduced into the above-described filter device to recover nucleated cells captured by the above-described filter material, and the recovered nucleated cell-containing solution is further centrifuged to eliminate the supernatant thereof, so as to concentrate the nucleated cells.

[Claim 11] The method for preparing a cell concentrate according to any one of claims 1 to 10, which is characterized in that the above-described filter material is configured such that a container having at least an inlet and an outlet for a cell-containing solution is packed with a nucleated cell-capturing material and a recovery solution-rectifying material, which consist of porous bodies, in this order in a direction from the inlet side to the outlet side for a cell-containing solution, and in that the value obtained by dividing the effective filtration area of the above-described filter material by the thickness of the nucleated cell-capturing material packed is between 15 and 120 cm.

[Claim 12] A method for preparing a cell concentrate, which comprises: introducing a cell-containing solution that contains nucleated cells and unnecessary cells into a filter device comprising a filter material for substantially capturing the nucleated cells and for substantially giving passage to the unnecessary cells, so as to capture the nucleated cells by the above-described filter material and to discharge the unnecessary cells from the above device; and introducing a recovery solution into the above-described filter device, so as to recover the nucleated cells captured by the above-described filter material,

wherein the above-described method is characterized in that it uses a filter device formed by packing a container having an inlet and an outlet for a cell-containing solution with a filter material obtained by stacking a nucleated cell-capturing material and a recovery solution-rectifying material, which consist of porous bodies wherein the

value obtained by dividing the effective filtration area of the above-described filter material by the thickness of the nucleated cell-capturing material packed is between 15 and 120 cm, such that a nucleated cell-capturing material is located on the inlet side of a cell-containing solution, and in that the above method comprises introducing the cell-containing solution from the inlet therefor into the filter device, so as to capture nucleated cells by the filter material, discharging unnecessary cells from the above-described filter device, and introducing a recovery solution from the outlet side for a cell-containing solution, so as to recover the nucleated cells captured by the above-described filter material from the inlet side for the cell-containing solution.

[Claim 13] The method for preparing a cell concentrate according to claim 11 or 12, wherein an aggregate-capturing material is further packed to the cell-containing solution inlet side of the above-described nucleated cell-capturing material.

[Claim 14] The method for preparing a cell concentrate according to any one of claims 11 to 13, wherein the filter material is a non-woven fabric.

[Claim 15] The method for preparing a cell concentrate according to claim 14, which is characterized in that the nucleated cell-capturing material and the recovery solution-rectifying material, which consist of non-woven fabrics, are:

- (i) a nucleated cell-capturing material consisting of a non-woven fabric having an average fiber diameter between 1.1 and 3.0  $\mu\text{m}$  and a packing density between 0.1 and 0.3  $\text{g}/\text{cm}^3$ ; and
- (ii) a recovery solution-rectifying material consisting of a non-woven fabric having an average fiber diameter between 0.5 and 1.5  $\mu\text{m}$  and a packing density between 0.1 and 0.3  $\text{g}/\text{cm}^3$ , and

which is characterized in that the average fiber diameter of the recovery solution-rectifying material is smaller than that of the nucleated cell-capturing material.

[Claim 16] The method for preparing a cell concentrate according to any one of claims 11 to 13, wherein the filter material is a sponge-like structure.

[Claim 17] The method for preparing a cell concentrate according to claim 16, which is characterized in that the nucleated cell-capturing material and the recovery solution-rectifying material, which consist of sponge-like structures, are:

- (i) a nucleated cell-capturing material consisting of a sponge-like structure having an average pore diameter during packing between 7 and 25  $\mu\text{m}$  and a porosity during packing between 55% and 90%; and
- (ii) a recovery solution-rectifying material consisting of a sponge-like structure having an average pore diameter during packing between 2 and 10  $\mu\text{m}$  and a porosity during packing between 55% and 90%, and

which is characterized in that the average pore diameter of the recovery solution-rectifying material is smaller than that of the nucleated cell-capturing material.

[Claim 18] The method for preparing a cell concentrate according to any one of claims 11 to 13, wherein the filter material is formed by the combination of a non-woven fabric with a sponge-like structure.

[Claim 19] A method for preserving cells in a frozen state, which further comprises the following steps, following the method for preparing a cell concentrate according to any one of claims 1 to 18:

- (b) a cell storage step of transferring the recovered nucleated cells to a storage bag having a storage unit and a cryopreservation unit via a nucleated cell-introducing port established at the above-described storage unit, and storing them;
- (c) a cell concentration step of centrifuging the nucleated cells stored in the above-described storage bag, while the cryopreservation unit is disposed at a position that is far from a rotation axis on a radius of gyration, so as to transfer the nucleated cells to the cryopreservation unit in the storage bag;
- (d) a container separation step of hermetically sealing the concentrated nucleated cells in the cryopreservation unit in a state where it contains no air, so as to separate the cryopreservation unit by melting down; and
- (e) a cryopreservation step of freezing the cryopreservation unit, in which the nucleated cells have been hermetically sealed, and preserving them,

wherein all of the above-described steps (b) to (e) are carried out in a hermetically sealed system.

[Claim 20] A method for preserving cells in a frozen state, which comprises at least the following steps (a) to (e):

- (a) a step of preparing a cell concentrate, which comprises introducing a cell-containing solution that contains nucleated cells and unnecessary cells into a filter device comprising a filter material for substantially capturing the nucleated cells and for substantially giving passage to the unnecessary cells, so as to capture the nucleated cells by the above-described filter material and to discharge the unnecessary cells from the above device, and introducing a recovery solution into the above-described filter device, so as to recover the nucleated cells captured by the above-described filter material;
- (b) a cell storage step of transferring the recovered nucleated cells to a storage bag having a storage unit and a cryopreservation unit via a nucleated cell-introducing port established at the above-described storage unit, and storing them;
- (c) a cell concentration step of centrifuging the nucleated cells stored in the above-described storage bag, while the cryopreservation unit is disposed at a position

that is far from a rotation axis on a radius of gyration, so as to transfer the nucleated cells to the cryopreservation unit in the storage bag;

(d) a container separation step of hermetically sealing the concentrated nucleated cells in the cryopreservation unit in a state where it contains no air, so as to separate the cryopreservation unit by melting down; and

(e) a cryopreservation step of freezing the cryopreservation unit, in which the nucleated cells have been hermetically sealed, and preserving them,

wherein the above-described steps (b) to (e) are carried out in a hermetically sealed system.

[Claim 21] The method for preserving cells in a frozen state according to claim 19 or 20, wherein the above-described unit for storing nucleated cells has a shape having an enlarged area, the cross section of which is gradually enlarged from the cryopreservation unit via a narrowed area.

[Claim 22] The method for preserving cells in a frozen state according to any one of claims 19 to 21, wherein, in the storage unit for storing the above-described nucleated cells, the narrowed area is used as a meltdown separation unit in the container separation step described in (d) above, and the cryopreservation unit is used as a container for cryopreservation of the nucleated cells in the cryopreservation step described in (e) above.

[Claim 23] The method for preserving cells in a frozen state according to any one of claims 19 to 22, wherein the cryopreservation unit of the above-described nucleated cells has a discharge port for the nucleated cells.

[Claim 24] The method for preserving cells in a frozen state according to any one of claims 19 to 23, wherein the cryopreservation unit of the above-described nucleated cells and/or the storage unit of the above-described nucleated cells have a cryoprotective agent-introducing unit.

[Claim 25] The method for preserving cells in a frozen state according to any one of claims 19 to 24, wherein the storage unit of the above-described nucleated cells has a filter device for discharging air contained in the bag for storing the nucleated cells.

[Claim 26] The method for preserving cells in a frozen state according to any one of claims 19 to 25, wherein the storage unit of the above-described nucleated cells has a conduit for discharging air contained in the bag for storing the nucleated cells, separately from a conduit for the nucleated cells.

[Claim 27] The method for preserving cells in a frozen state according to any one of claims 19 to 26, wherein a cryoprotective agent is added from the above-described cryoprotective agent-introducing unit before the container separation step described in

(d) above.

[Claim 28] The method for preserving cells in a frozen state according to any one of claims 19 to 27, wherein a cryoprotective agent is added from the above-described cryoprotective agent-introducing unit after the container separation step described in (d) above.

[Claim 29] A method for producing a frozen cell product, which uses the method according to any one of claims 19 to 28.

[Claim 30] A cell composition, which is obtained by the method according to any one of claims 1 to 18.

[Claim 31] The cell composition according to claim 30, which is obtained by further centrifuging the above-described cell composition and eliminating a supernatant, so as to concentrate nucleated cells.

[Claim 32] The cell composition according to claim 30 or 31, wherein the above-described unnecessary cells are erythrocytes.

[Claim 33] The cell composition according to claim 30 or 31, wherein the above-described nucleated cells are hematopoietic stem cells.

[Claim 34] A frozen cell product, which is prepared by the method according to any one of claims 19 to 28.